Systemic and portal hemodynamic effects of anandamide

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Garcia, Nelson Jr., Zoltán Járai, Faridoddin Mirshahi, George Kunos, and Arun J. Sanyal. Systemic and portal hemodynamic effects of anandamide. *Am J Physiol Gastrointest Liver Physiol* 280: G14–G20, 2001.—The endogenous cannabinoid anandamide causes hypotension and mesenteric arteriolar dilation. A detailed analysis of its effects on systemic and portal venous hemodynamics had not yet been performed. We assessed the effects of anandamide (0.4–10 mg/kg) on systemic and portal hemodynamics with and without prior treatment with various antagonists. The specific antagonists used included SR-141716A, N^ω-nitro-L-arginine methyl ester, indomethacin, and nordihydroguaiaretic acid. Anandamide produced a dose-dependent decrease in mean arterial pressure due to a drop in systemic vascular resistance (SVR) that was accompanied by a compensatory rise in cardiac output. Anandamide also elicited an increase in both portal venous flow and pressure, along with a decline in mesenteric vascular resistance (MVR). Pretreatment with 3 mg/kg SR-141716A, a CB_1 antagonist, prevented the decline of SVR and MVR from the lower dose of anandamide. Antagonism of nitric oxide synthetase, cyclooxygenase, or 5-lipoxygenase did not prevent the systemic nor the portal hemodynamic effects of anandamide. Furthermore, the use of R-methanandamide, a stable analog of anandamide, produced similar hemodynamic effects on the mesenteric vasculature, thereby implying that the effects of anandamide are not related to its breakdown products. Anandamide produced profound, dose-dependent alterations in both the systemic and portal circulations that could be at least partially blocked by pretreatment with SR-141716A.

portal vein flow; portal vein pressure; cannabinoids; SR-141716A; portal hypertension; blood pressure; splanchnic blood flow; cirrhosis

THE REGULATION OF BLOOD FLOW through various circulatory beds is complex and incompletely understood. It is, however, appreciated that the blood flow and pressure in any given vascular bed depend on a balance between vasodilatory and vasoconstrictive factors. Over the last 10 years, nitric oxide (NO) has gained predominance as the major vasodilatory factor that controls vascular tone (19). However, it is possible as well as probable that other vasodilatory pathways also exist that may affect both regional and systemic blood flow.

In 1992, the first endogenous cannabinoid was isolated and identified as arachidonyl ethanolamide (anandamide) (6). Recent studies (23, 24), have shown that anandamide produces hypotension and bradycardia in anesthetized rats. It has also been shown to cause mesenteric arterial dilation in isolated mesenteric arterial preparations (28). Theoretically, increased mesenteric arterial dilation could lead to increased portal venous flow (PVF) and portal venous pressure (PVP) (10). However, the effects of anandamide on portal hemodynamics have not been previously defined. Also, other than the effects of anandamide on heart rate and blood pressure, a detailed analysis of the systemic hemodynamic effects of anandamide have not been performed.

The objective of the present study was to perform a detailed characterization of the effects of anandamide on the portal and systemic circulation in normal, anesthetized rats. This was done by simultaneous measurement of heart rate, blood pressure, cardiac output (CO), systemic venous pressure, mesenteric arterial flow, PVF, and PVP.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–350 g were used for all experiments. The animals were maintained on a 12:12-h light/dark cycle and allowed rat chow and water ad libitum in the animal care facility at the Virginia Commonwealth University-Medical College of Virginia. All animals were allowed to acclimate for several days before surgery. All studies were approved and performed in compliance with the guidelines of the institutional animal use and care committee.

Experimental technique. Anesthesia was induced with the intraperitoneal administration of pentobarbital sodium at a dose of 50 mg/kg. The animal was then placed on a homeotherm blanket (Harvard Apparatus, South Natick, MA) to maintain body temperature between 37.0 and 38.0°C. Once anesthesia was induced, the right femoral vein was cannulated with PE-50 tubing (Becton Dickinson, Franklin Lakes, NJ), and 0.9% NaCl was infused intravenously at a rate of $0.02 \text{ cm}^3 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ by a syringe pump (Razel Scientific Instruments, Stamford, CT) to compensate for evaporative losses due to the surgical preparation. A fluid-filled PE-50 tube was

used to cannulate the right internal jugular vein and served as the route of drug administration as well as the route for administration of saline for transpulmonary thermodilution measurements of CO. Supplemental pentobarbital sodium (10 mg/kg) was given intravenously as indicated on the basis of spontaneous muscle activity or lessening of the anesthetic plane. A tracheostomy was performed using PE-190 tubing to maintain airway patency.

For measurement of systemic blood pressure, a fluid-filled PE-50 tube was inserted into the right femoral artery and connected to a pressure transducer (Transpac; Abbott Laboratories, North Chicago, IL). A thermistor-tip catheter (9030-12; Columbus Instruments, Columbus, OH) was inserted into the left carotid artery and positioned at the level of the aortic arch for measurement of CO.

Next, a laparotomy was performed using a transverse abdominal incision at approximately the level of the porta hepatis. The ileocolic vein was identified and traced to the mesenteric border of the small intestine. A tributary of the ileocolic vein that was more or less in a direct line with the ileocolic vein was identified and cannulated using a 22-gauge, 1-in. catheter (Johnson and Johnson Medical, Arlington, TX). The catheter was then threaded toward the portal vein, thereby placing it within the portal vein without tying off the portal circulation. This catheter was also connected to a pressure transducer for pressure measurements. A 1.5 RB ultrasound transit time flow probe (Transonic Systems, Ithaca, NY) was placed around the portal vein at the level of the porta hepatis and on the mesenteric artery and connected to a small animal flow meter (T206; Transonic Systems). When all surgical procedures were completed, the abdominal wall was reapproximated and a 30-min stabilization period was allowed.

Drugs. Anandamide was obtained from Deva Biotech (Hatboro, PA). SR-141716A (CB$_1$ receptor antagonist) was obtained from the National Institute of Drug Abuse. N^ω-nitro-L-arginine methyl ester (L-NAME, an inhibitor of NO synthetase), indomethacin, and nordihydroguaiaretic acid (NDGA; 0.1 mg/kg; a 5-lipooxygenase inhibitor) were all obtained from Sigma (St. Louis, MO). The vehicle for all drugs except L-NAME consisted of emulphor:ethanol:saline 1:1:8. Emulphor is a polyoxyphenylated vegetable oil. L-NAME was prepared in 0.9% normal saline before each experiment.

Study design and experimental protocols. In the first set of experiments, at the end of the stabilization period, the animals received anandamide at varying doses (0.4, 4, and 10 mg/kg). Mean arterial pressure (MAP), central venous pressure (CVP), PVP, and PVF were measured continuously for 10 min following each dose. By using the transpulmonary thermodilution technique (3), CO was obtained at 1, 2, 3, 5, and 10 min after drug administration by injecting 150 μl of saline at room temperature into the right internal jugular vein. In selected animals, the superior mesenteric arterial flow was measured as well.

Following completion of the first set of studies, a second set of experiments was performed to determine the interactions between anandamide and other known regulators of vascular tone and blood flow. Following anesthesia and surgical preparation, animals received pretreatment with one of four drugs administered 10 min before administration of anandamide (4 or 10 mg/kg): SR-141716A (3 mg/kg), L-NAME (6.25 mg/kg), indomethacin (5 mg/kg), or NDGA (0.1 mg/kg).

Data acquisition and storage. All pressure transducers were connected to a analog-digital converter (Transonic Systems) that had up to 16 independent ports. The zero point for all pressure transducers was set to the level of the right

heart. CO was measured using well-established thermodilution methods (3) by using a Cardiotherm 400-R instrument (Columbus Instruments, Columbus, OH) connected to the thermodilution catheter in the carotid arch. PVF and mesenteric arterial flow were directly measured using the T206 small animal flow meter. All data were recorded simultaneously in real time on a Pentium II personal computer using Windaq data acquisition software (Dataq Instruments, Akron, OH). Data were stored in electronic files using the Windaq software.

Calculations and data analysis. MAP was calculated using the Windaq software by obtaining the average of the arterial waveform over a 3-s period. Systemic vascular resistance (SVR) was calculated from: [(MAP − CVP)/CO]·80 (15). Mesenteric vascular resistance (MVR) was similarly calculated as [(MAP − PVP)/PVF]·80. Data from Windaq software were extracted onto a Microsoft Excel 7.0 spreadsheet for individual experiments, and summary data from multiple experiments were analyzed on a separate spreadsheet. Statistical analyses were performed using both Microsoft Excel 7.0 and SPSS software (SPSS, Chicago, IL). A P value <0.05 was considered significant.

RESULTS

Detailed analyses of systemic and splanchnic hemodynamics were performed in an initial set of five rats. Before each experiment, all pressure transducers were calibrated against a sphygmomanometer, and the flowmeter was calibrated via an internal calibration system per the manufacturer's instructions. Before initiation of each experiment, stable normal baselines were documented in each case for at least 20 min. Raw data measurements were made simultaneously, as shown in Fig. 1.

Effects of anandamide on systemic hemodynamics. Anandamide produced a profound, dose-dependent drop in arterial pressure within 2 min of administration. The effects of anandamide on systemic hemodynamics are shown in Fig. 2. Although 0.4 mg/kg of anandamide had relatively minor effects on the MAP (Fig. 2*A*), both 4 and 10 mg/kg produced a marked, significant decrease in MAP [$P = 0.008$ for 4 mg/kg and $P = 0.01$ for 10 mg/kg by paired *t*-test (baseline vs. maximal decrease)]. The effects of anandamide were most pronounced within the first 1–3 min following intravenous administration, and the MAP returned to baseline values within 10–15 min. A transient pressor response, as noted previously (23), lasting <1 min after administration, was seen particularly with the higher dose of anandamide (10 mg/kg).

The principal contributor to the hypotensive effects of anandamide was a decrease in SVR (Fig. 2*B*). Although only a small, transient decrease in SVR corresponding to the minor drop in arterial pressure was seen with 0.4 mg/kg of anandamide, there was a significant, dose-dependent drop in SVR at higher doses [$P = 0.008$ for 4 mg/kg and $P = 0.02$ for 10 mg/kg by paired *t*-test (baseline vs. maximal decrease)]. The temporal profile of these changes matched those in arterial pressure, and the two plots were practically superimposable (Fig. 2, *A* and *B*).
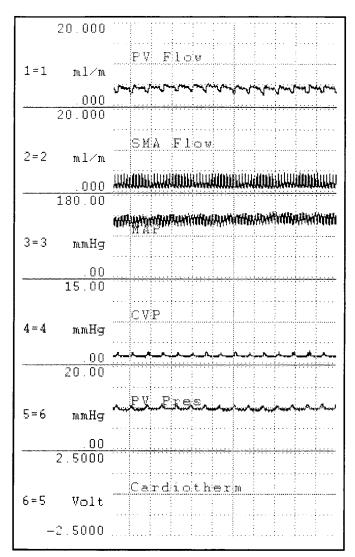
Fig. 1. A tracing of simultaneously obtained data on portal vein (PV) flow (*channel 1*), superior mesenteric arterial (SMA) flow (*channel 2*), systemic mean arterial pressure (MAP; *channel 3*), central venous pressure (CVP; *channel 4*), PV pressure (*channel 5*), and cardiac output measurement (Cardiotherm; *channel 6*).

The hypotensive effect of anandamide was accompanied by a small transient increase in CO (Fig. 2C). Direct measurements were made at 0 (baseline), 1, 2, 3, 5, and 10 min. At the lowest dose (0.4 mg/kg), there were no significant changes in CO compared with baseline values ($P = 0.3$). However, at higher doses, the CO increased compared both with baseline [$P = 0.05$ and 0.02 for 4 and 10 mg/kg anandamide, respectively, by paired t-test (baseline vs. maximal increase)] and with the lowest dose ($P = 0.04$ by ANOVA). The time course of the changes in CO were similar to those in arterial pressure and probably reflected a compensatory increase in CO secondary to the arterial hypotension.

The increase in CO was mainly due to an increase in stroke volume (CO/heart rate). As noted previously (23, 24), anandamide produced a bradycardia but it was not sustained. This, however, contributed only partly to the increase in stroke volume, indicating that there

was a true increase in the volume of blood pumped by the heart. The changes in systemic venous pressures were quite variable from animal to animal and ranged from either no change to a small decrease in pressure. These variable changes in venous pressure probably reflected both the changes in venous return due to peripheral pooling and/or changes in right heart hemodynamics.

The effects of anandamide on the portal venous circulation. At baseline, PVP varied from 6.29 to 8.9 mmHg, whereas PVF varied from 5.31 to 9.01 ml/min,
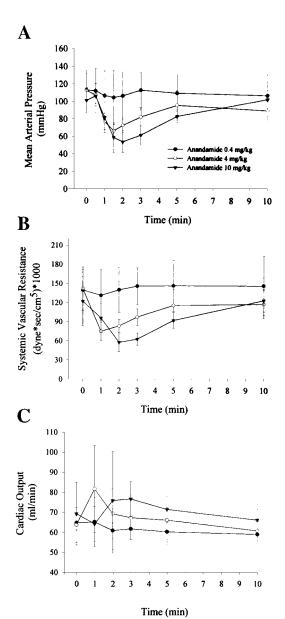

Fig. 2. The effects of anandamide (0.4–10 mg/kg) administered as an intravenous bolus on the MAP (*A*), systemic vascular resistance (SVR) (*B*), and cardiac output (CO) (*C*). Although a small decrease in SVR without a significant change in MAP or CO occurred after 0.4 mg/kg of anandamide, a dose-dependent decrease in SVR and MAP occurred at higher doses, along with an increase in CO ($P < 0.05$, both for baseline compared with maximal changes and effects of 0.4 mg/kg compared with higher doses). The means ± SD of data from 5 animals are shown.

which are well within published values (8, 20, 21). Anandamide produced a dose-dependent increase in PVF and PVP (Fig. 3, *A* and *B*). In contrast to its systemic effects (Fig. 2), an increase in PVF was seen at even the lowest dose of anandamide (0.4 mg/kg). This was even more pronounced at higher doses of anandamide. These effects were short-lived, and PVF returned to baseline within 5 min. The increase in PVF was accompanied by an increase in PVP. However, at all doses, the percent increment in PVF was almost twice that seen for PVP.
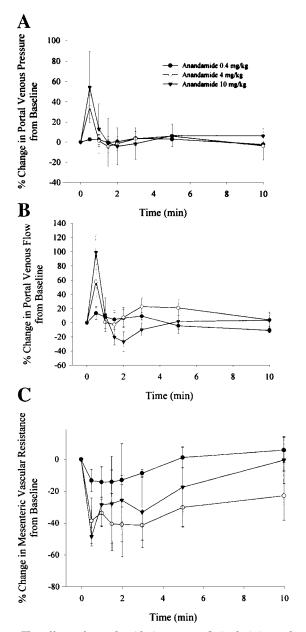

Fig. 3. The effects of anandamide (0.4–10 mg/kg) administered as an intravenous bolus on percent change in PV pressure (PVP) (*A*), PV flow (PVF) (*B*), and mesenteric vascular resistance (MVR) (*C*). Anandamide produced a short-lived, dose-dependent increase in PVF and PVP due to a decline in the MVR. The effects with both 4 and 10 mg/kg of anandamide were significantly greater ($P < 0.05$) than those with 0.4 mg/kg of anandamide. The means $\pm$ SD of data from 5 animals are shown.

The changes in PVF and PVP were due to a decrease in MVR (Fig. 3*C*). Data obtained with either PVF or mesenteric arterial flow were qualitatively similar. These data demonstrate that anandamide has a dose-dependent mesenteric arterial vasodilatory effect, resulting in an increase in PVF and PVP.

Effects of pretreatment with specific antagonists. To test the possibility that the anandamide effects were mediated, at least partially, by either NO or prostaglandins, the effects of anandamide were restudied in another set of animals ($n = 9$) following pretreatment with specific blocking antagonists (Fig. 4). In each given animal, pretreatment with only a single antagonist was done. The hypotensive effects of anandamide were not significantly blocked by L-NAME, indomethacin, or NDGA. In animals receiving L-NAME, there was an increase in resting MAP after L-NAME administration. However, on subsequent administration of anandamide, a dose-dependent hypotensive effect was obtained.
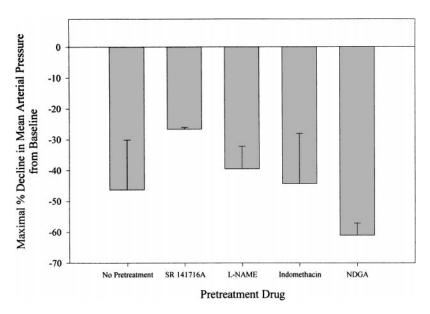
The hypotensive effects of anandamide could be blocked by SR-141716A. The blockade was nearly complete at 4 mg/kg of anandamide, whereas only 42% of the hypotensive effect at 10 mg/kg of anandamide could be blocked by a fixed dose of the antagonist (3 mg/kg). This was due to blockade of the effects of anandamide (4 mg/kg) on SVR and MVR (Fig. 5). In contrast, compared with non-pretreated animals, SR-141716A did not block the decrease in SVR produced by 10 mg/kg of anandamide. Interestingly, despite an equivalent drop in SVR compared with animals that did not receive SR-141716A, a commensurate drop in MAP was not observed after 10 mg/kg anandamide (Fig. 3) in animals pretreated with SR 141716A. This was due to increased CO, which lowered the SVR and tended to maintain the MAP in pretreated animals. Pretreatment with SR-141716A also did not affect the mesenteric arterial vasodilatory effects of 10 mg/kg anandamide, as indicated by a failure to block the anandamide-induced decrease in MVR.

To ascertain whether the observed effects with anandamide were due to anandamide itself or to its breakdown products, the studies were repeated with R-methanandamide, a long-acting stable analog of anandamide (1). Methanandamide also produced similar effects. Although the systemic hypotensive effects could be blocked by SR-141716A, the portal hemodynamic effects could not be blocked. These data indicate that the mesenteric arteriolar dilation produced by anandamide is not related to its breakdown products. Also, the ability of SR-141716A to block the portal hypertensive effects of 4 but not 10 mg/kg anandamide or R-methanandamide indicates that the CB_1 receptor as well as other pathways are involved in mediating these effects.

DISCUSSION

The hypotensive effects of tetrahydrocannabinol (THC), the major psychoactive component of marijuana, were established almost 30 years ago (7). More

Fig. 4. The effects of pretreatment with various antagonists on maximal percentage decline in MAP before administration of 10 mg/kg anandamide are shown. N^ω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthetase; indomethacin, a cyclooxygenase inhibitor; and nordihydroguaiaretic acid (NDGA), a 5-lipooxygenase inhibitor, had no significant blocking effects. In contrast, SR-141716A, a CB_1 receptor antagonist, produced an ~40% blockade of the hypotensive effect of anandamide. Means ± SD from 9 animals are shown.
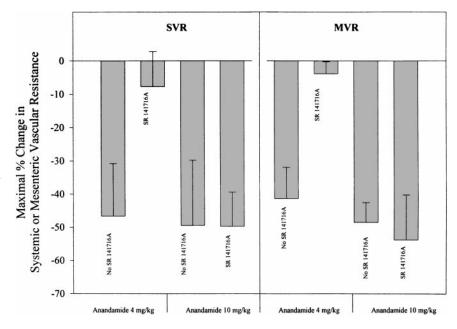
recently, these properties were also described for anandamide (24), an endogenous ligand for cannabinoid receptors (6). The present study corroborated previous findings (23, 24) that anandamide produced a profound systemic hypotension and further demonstrated that the hypotensive effects of anandamide were due to a decrease in SVR.

The effects of anandamide on SVR most likely represented a composite of its effects on various regional circulatory beds. For example, THC (2, 18) has been shown to increase cerebral circulation, and anandamide has been found to increase renal blood flow by producing afferent arteriolar dilation (5). It is also recognized that mesenteric arterioles are dilated by anandamide (22, 28). However, the effects of anandamide-induced mesenteric arteriolar dilation on portal venous hemodynamics had not been previously characterized.

An important observation in the present study was that anandamide increased both PVF and PVP. It is interesting to note that the degree in increment in PVF did not translate into a similar degree of rise in PVP. A potential explanation for this observation could be that anandamide decreases resistance to flow through the liver at the level of the hepatic sinusoids. Such an effect would dampen the rise in PVP due to an increase in PVF. Indeed, we have recently demonstrated the presence of CB_1 receptor mRNA (16) on both normal and cirrhotic hepatic sinusoidal endothelium, suggesting that potential targets for endogenous cannabinoids exist in the hepatic microcirculation. However, this hypothesis remains to be experimentally verified.

Fig. 5. The effects of pretreatment with SR-141716A, a specific CB_1 receptor antagonist, on SVR and MVR. SR-141716A (3 mg/kg) blocked the decrease in SVR and MVR produced by 4 mg/kg anandamide. However, when 10 mg/kg anandamide was used, no significant effects of either SVR or MVR were noted. Means ± SD from 5–7 animals are shown.

The nearly complete blockade of low dose (4 mg/kg) anandamide-induced decreases in SVR, MVR, and MAP as well as the increase in PVF and PVP by SR-141716A indicated that these effects were mediated via CB_1 receptors. These data are in agreement with the loss of anandamide-induced hypotension in CB_1 receptor knockout mice (14). Also, the preservation of the hypotensive effects with R-methanandamide further demonstrated that the observed effects were due to anandamide itself rather than its breakdown products.

In contrast, the effects of high-dose anandamide were more intriguing. Pretreatment with SR-141716A had virtually no effect on the decrease in SVR or MVR produced by subsequent administration of anandamide (10 mg/kg). A potential explanation could be that this was due to a high dose of the agonist (anandamide) in the presence of a relatively low dose of the competitive antagonist (SR-141716A). If so, one would expect similar hemodynamic changes in pretreated vs. non-pretreated animals. The near-identical drop in SVR in pretreated animals compared with non-pretreated animals was, however, associated with only a limited drop in MAP after anandamide administration. This was mainly due to a greater increase in CO than that noted in non-pretreated animals, which tended to keep the SVR down and MAP up. The basis for these observations remains to be experimentally defined.

It has been suggested (23) that anandamide acts at a presynaptic location to decrease norepinephrine release by sympathetic neurons, thereby decreasing sympathetic vasoconstrictive tone and heart rate. Indeed, CB_1 receptors have also now been identified on sympathetic nerve fibers innervating resistance vessels (17). The hypotensive effects of the synthetic cannabinoids WIN-55212–2 and HU-210 are, however, retained in sympathetomized rats receiving norepinephrine infusions (26). Also, the maximal hypotension with HU-210 exceeds that seen after α-adrenergic blockade (13), indicating that additional peripheral vasodilatory effects are also present. The documentation of CB_1 receptors, anandamide, and the amidases that break down anandamide in endothelial cells and renal tissue (5) supports such a concept.

Recent studies have focused on the role of both direct cannabinoid receptor-mediated effects on vascular smooth muscle cells (12) and indirect endothelium-dependent effects (5, 28). Binding of anandamide to CB_1 receptors has been shown to increase NO in renal vasculature (5). Although the present studies could not demonstrate blockade of anandamide-induced hypotension by L-NAME, this does not completely exclude the possibility that endothelial CB_1 receptors mediate localized NO-dependent vasodilation in some regional vascular beds.

A key question relates to the potential role of anandamide and other endogenous cannabinoids under normal and disease states. The failure to demonstrate major hemodynamic effects following administration of SR-141716A may not necessarily be construed to indicate a lack of substantive effects in normal rats. For example, it has been proposed that, following a meal, a rise in interstitial Ca^{2+} concentration causes release of anandamide from perivascular nerve terminals (4, 11), which can then produce vasodilation by both endothelium-dependent, SR-141716A-sensitive and endothelium-independent, SR-141716A-insensitive pathways. A potential mechanism for the latter pathway may involve interaction with capsaicin-sensitive vanilloid receptors on sensory nerve terminals, which cause release of calcitonin gene-related peptide (CGRP) and cause CGRP-mediated vasodilation (29). The current study was not designed to examine such effects, and these now require further research.

It has also been shown that exposure to endotoxin (*Escherichia coli* lipopolysaccharide) stimulates release of both anandamide and 2-arachidonoyl glycerol from platelets and macrophages (25). Under conditions in which platelets and/or macrophages are activated, it is possible that the release of anandamide may cause vasodilation and contribute to hypotension. Indeed, anandamide has been shown to contribute to severe irreversible hemorrhagic shock (27). Theoretically, the release of anandamide may also contribute to the local vasodilation seen in areas of inflammation as well as the mesenteric and systemic arterial vasodilation present in cirrhosis (9). Although much additional work is necessary, the current studies provide an important basic step in the right direction and should provide the groundwork for future studies to define the role of endogenous cannabinoids under normal and pathological circumstances.

REFERENCES

1. **Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, and Makriyannis A.** (R)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J Med Chem* 37: 1889–1893, 1994.
2. **Bloom AS, Tershner S, Fuller SA, and Stein EA.** Cannabinoid-induced alterations in regional cerebral blood flow in the rat. *Pharmacol Biochem Behav* 57: 625–631, 1997.
3. **Brackett DJ, Schaefer CF, Tompkins P, Fagraeus L, Peters LJ, and Wilson MF.** Evaluation of cardiac output, total peripheral vascular resistance, and plasma concentrations of vasopressin in the conscious, unrestrained rat during endotoxemia. *Circ Shock* 17: 273–284, 1985.
4. **Bukoski RD, Bian K, Wang Y, and Mupanomunda M.** Perivascular sensory nerve Ca^{2+} receptor and Ca^{2+}-induced relaxation of isolated arteries. *Hypertension* 30: 1431–1439, 1997.
5. **Deutsch DG, Goligorsky MS, Schmid PC, Krebsbach RJ, Schmid HH, Das SK, Dey SK, Arreaza G, Thorup C, Stefano G, and Moore LC.** Production and physiological actions of anandamide in the vasculature of the rat kidney. *J Clin Invest* 100: 1538–1546, 1997.
6. **Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, and Mechoulam R.** Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949, 1992.
7. **Dewey WL, Harris LS, Howes JF, Kennedy JS, Granchelli FE, Pars HG, and Razdan RK.** Pharmacology of some marijuana constituents and 2 heterocyclic analogues. *Nature* 226: 1265–1267, 1970.

8. **Fernandez M, Garcia-Pagan JC, Casadevall M, Mourelle MI, Pique JM, Bosch J, and Rodes J.** Acute and chronic cyclooxygenase blockage in portal-hypertensive rats: influence in nitric oxide biosynthesis. *Gastroenterology* 110: 1529–1535, 1996.

9. **Groszmann RJ.** Hyperdynamic state in chronic liver diseases. *J Hepatol* 17, *Suppl* 2: S38–S40, 1993.

10. **Gupta TK, Chen L, and Groszmann RJ.** Pathophysiology of portal hypertension. *Clin Liver Dis* 1: 1–12, 1997.

11. **Ishioka N and Bukoski RD.** A role for *N*-arachidonyletha-nolamine (anandamide) as the mediator of sensory nerve-dependent Ca^{2+}-induced relaxation. *J Pharmacol Exp Ther* 289: 245–250, 1999.

12. **Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, Zimmer AM, Bonner TI, Buckley NE, Mezey E, Razdan RK, Zimmer A, and Kunos G.** Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci USA* 96: 14136–14141, 1999.

13. **Lake KD, Compton DR, Varga K, Martin BR, and Kunos G.** Cannabinoid-induced hypotension and bradycardia in rats mediated by CB1-like cannabinoid receptors. *J Pharmacol Exp Ther* 281: 1030–1037, 1997.

14. **Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, and Parmentier M.** Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 283: 401–404, 1999.

15. **Lee SS, Girod C, Valla D, Geoffroy P, and Lebrec D.** Effects of pentobarbital sodium anesthesia on splanchnic hemodynamics of normal and portal-hypertensive rats. *Am J Physiol Gastrointest Liver Physiol* 249: G528–G532, 1985.

16. **Liu J, Mirshahi F, Sanyal AJ, Khanolkar AD, Makriyannis A, and Kunos G.** Functional CB1 cannabinoid receptors in human vascular endothelial cells. *Biochem J* 346: 835–840, 2000.

17. **Malinowska B, Godlewski G, Bucher B, and Schlicker E.** Cannabinoid CB1 receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. *Naunyn Schmiedebergs Arch Pharmacol* 356: 197–202, 1997.

18. **Mathew RJ, Wilson WH, Coleman RE, Turkington TG, and Degrado TR.** Marijuana intoxication and brain activation in marijuana smokers. *Life Sci* 60: 2075–2089, 1997.

19. **Moncada S and Higgs A.** The L-arginine-nitric oxide pathway. *N Engl J Med* 329: 2002–2012, 1993.

20. **Oberti F, Sogni P, Cailmail S, Moreau R, Pipy B, and Lebrec D.** Role of prostacyclin in hemodynamic alterations in conscious rats with extrahepatic or intrahepatic portal hypertension. *Hepatology* 18: 621–627, 1993.

21. **Pannen BH and Bauer M.** Differential regulation of hepatic arterial and portal venous vascular resistance by nitric oxide and carbon monoxide in rats. *Life Sci* 62: 2025–2033, 1998.

22. **Randall MD, McCulloch AI, and Kendall DA.** Comparative pharmacology of endothelium-derived hyperpolarizing factor and anandamide in rat isolated mesentery. *Eur J Pharmacol* 333: 191–197, 1997.

23. **Varga K, Lake KD, Huangfu D, Guyenet PG, and Kunos G.** Mechanism of the hypotensive action of anandamide in anesthetized rats. *Hypertension* 28: 682–686, 1996.

24. **Varga K, Lake K, Martin BR, and Kunos G.** Novel antagonist implicates the CB1 cannabinoid receptor in the hypotensive action of anandamide. *Eur J Pharmacol* 278: 279–283, 1995.

25. **Varga K, Wagner JA, Bridgen DT, and Kunos G.** Platelet- and macropahage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. *FASEB J* 12: 1035–1044, 1998.

26. **Vidrio H, Sanchez-Salvatori MA, and Medina M.** Cardiovascular effects of (−)-11-OH-delta 8-tetrahydrocannabinol-dimethylheptyl in rats. *J Cardiovasc Pharmacol* 28: 332–336, 1996.

27. **Wagner JA, Varga K, Ellis EF, Rzigalinski BA, Martin BR, and Kunos G.** Activation of peripheral CB1 cannabinoid receptors in haemorrhagic shock. *Nature* 390: 518–521, 1997.

28. **Wagner JA, Varga K, Jarai Z, and Kunos G.** Mesenteric vasodilation mediated by endothelial anandamide receptors. *Hypertension* 33: 429–434, 1999.

29. **Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di M, Julius DV, and Hogestatt ED.** Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400: 452–457, 1999.